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Impact of macrophages on *Balamuthia mandrillaris* virulence properties using human brain microvascular endothelial cells in vitro

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Background: *Balamuthia* amoebic encephalitis (BAE) is a serious human disease (caused by *Balamuthia mandrillaris*) almost always leading to death. An important step in BAE is amoebae invasion of the bloodstream, followed by their haematogenous spread. *Balamuthia mandrillaris* entry into the central nervous system (CNS) most likely occurs at the blood–brain barrier (BBB) sites. Macrophages are thought to be the first line of defense in many infectious diseases and are present in high numbers during infections. The objective of the present study was to determine the impact of cytokines and macrophages on the virulence characteristics of *B. mandrillaris in vitro*.

Methods: *In vitro*, *B. mandrillaris* were used to demonstrate the effects of cytokines and macrophages on the physiological and morphological characteristics of amoeba. Using human brain microvascular endothelial cells (HBMEC), which constitutes the blood–brain barrier, adhesion and cytotoxicity assays were performed. To investigate the engulfing property of the amoeba, phagocytosis assays were performed using fluorescein isothiocyanate (FITC) labeled *E. coli* K12. Moreover zymography assay were also used to observe the proteolytic activity of amoeba.

Results: It was observed *B. mandrillaris* exhibited >90% binding and >70% cytotoxicity to HBMEC which was further enhanced in the presence of cytokines and macrophages. It has also been observed that cytokines TNF-α and TGF-β significantly increased the *B. mandrillaris* numbers in the presence of macrophages. It is important to note that amoebic numbers were more than doubled in the presence of cytokines and macrophages within 24h. We have shown in the past the bacteria uptake by *B. mandrillaris* is limited which is further significantly inhibited in the presence of cytokines during phagocytosis assays. Zymography assays revealed that cytokines and macrophages have no inhibitory effect on proteolytic activity of *B. mandrillaris*. In addition the activated macrophages also could not show any vital inhibitory effects on amoebic virulence properties.

Conclusion: Overall we described for the first time that cytokines and macrophages has no inhibitory effects on the virulence properties of *B. mandrillaris in vitro*.

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Association of ABO blood groups and complicated *Plasmodium falciparum* malaria in Accra, Ghana

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Background: The clinical outcome of *Plasmodium falciparum* malaria in endemic areas is associated with erythrocyte polymorphisms including the ABO blood groups. Studies have reported association of ABO blood group to susceptibility, resistance and severity of P. falciparum malaria infection. Individuals with blood group 'A' have been found to be highly susceptible to falciparum malaria whereas blood group 'O' is said to confer protection against complicated cases.

Methods: The study was conducted between January to April 2010, at the outpatient department of the Korle-Bu teaching Hospital in Accra, Ghana. Five milliliters of blood sample was collected from each participant and the haemoglobin level, parasitaemia and ABO blood group of the samples collected were determined.

Results: We analysed samples from 239 malaria patients and found that group O was present in 16.1% of complicated cases weighed against 40.9% of uncomplicated controls. Individuals with complicated malaria were about twice likely to be of blood group A and B than O (A vs. O, OR=1.90, 95% CI=1.59 – 2.26, P<0.0001; B vs. O, OR=1.82. 95% CI=1.57 – 2.23, P<0.0001). Blood group O participants with complicated diseases had low parasitaemia compared to the blood groups (P<0.0001). This may give blood group O individuals a survival advantage over the other groups in complicated malaria as suggested. Participants with complicated falciparum malaria were generally anaemic and younger than those with uncomplicated disease

Conclusion: Blood group O offers some protection from complicated falciparum malaria and may possess a survival advantage over the Non-O groups.

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Genotyping of *Plasmodium falciparum* in Bangladesh using Antigenic Polymorphic markers and comparison with anti-malarial drug resistance markers genotype

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Background: Polymerase-Chian-reaction (PCR)- Restriction Fragments –length polymorphisim and Tag-man real-time PCR assay used for molecular characterization of Plasmodium falci-

parum isolates using antigenic polymorphic and drug resistance markers.

Methods: 33 pairs samples were collected from two clinical studies, assessing anti-malarial drug resistance in the Chittagong Hill tracts Bangladesh during 2004 and 2005. Blood samples were taken at Day 0 and at the day of recurrent parasitemia during 42 days follow-up and analyzed using PCR-RFLP and Tag-man real-time PCR assay.

Results: We found high parasite diversity in the population and polyclonal infection in the host using MSP-1 and GLURP loci. . MSP-1 allelic family K1 was positive in 19 (58%) with, 6 different fragments (150−280 bp)., MAD20 was detected in 25 (76%) with 8 different fragments (130-300 bp) and RO33 allele was detected in 21 (64%) samples with 4 different sizes. The region II of GLURP was present in all 33 (100%) samples with 6 different fragments (600−1100 bp). MAD-20 showed higher numbers of PCR positivity with higher numbers of allele. Molecular analysis of anti-malarial drug resistance marker such as pfcrt and pfmdr-1by PCR- RFLP showed high prevalence of mutan tpfcrt76T (90%) and (80%) of wild pfmdr1N86. Among the 33 paired samplespfmdr1represent 18.2% were heterozygous (N86Y) and 18.2% were mutant (86Y).

In the pre-treatment isolates 6 (18%) were CVMNK haplotype and all mixed with SVMNT haplotype. A total of 5 (83%) out of 6 were mixed with CVIET haplotype. In all 33 pre-treatment isolates SVMNT haplotype and 32 of them (97%) CVIET haplotype. In the post-treatment samples 10 (30%) isolates were CVMNK haplotype, 30 (91%) SVMNT haplotype and 24 (73%) CVIET haplotype, all of them mixed with SVMNT haplotype.

Conclusion: Genotype using antigenic polymorphic and anti-malarial drug resistance markers can be limit misclassification of recurrent parasitemia Analysis of K76T point mutation by PCR-RFLP and real-time PCR showed that both methods are equally proficient in detecting K76 and 76T alleles but Taq-man multiplex real-time is significantly better in detecting both alleles K76 and K76T (uncorrected X2 = 4.12, P = .042).

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Cultivation and molecular characterization of blastocystis spp. by polymerase chain reaction

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Background: Blastocystis *is* often reported as a causative agent of chronic diarrhoea in Pakistan. The objective of this study was isolation and culturing of Blastocystis spp obtained from diarrhoeal patients and poultry by using different cultural media. Molecular identification was done by PCR.

Methods: Three hundred and fifty fecal samples were collected from patients with gastrointestinal symptoms and their clinical histories were recorded. Direct microscopy and concentration method were used for the detection of parasites and Kinyoun method for Cryptosporidium. Three hundred and fifty poultry faecal samples were also collected and Blastocystis spp was detected by direct microscopy. Positive samples of Blastocystis spp were cultured on Jone's' medium supplemented with 10% horse serum and Locke-egg (LE) medium. PCR was used for molecular characterization of Blastocystis.

Results: Results indicated that out of 350 faecal samples parasites were present in 40% of cases. Blastocystitis spp was present in 48% followed by *Entamoeba histolytica* (24%), Cryptosporidium spp (15%), *Giardia lamblia* (12%), *Ascaris lumbricoides* (1%). Single pathogen infection was present in 80% and in 20% of the cases infection was in combination with other parasites. The most frequent symptoms were abdominal pain in 50% of the cases, diarrhoea in 26%, fever in 16% and vomiting in 8% of the cases.

Out of 350 poultry samples (21%) were positive for Blastocystis spp. 50 positive samples of Blastocystis spp. from patients and 20 poultry samples cultured in vitro on Jone's media indicated no growth of *Blastocystis* spp. while on Locke-egg (LE) medium, heavy growth was obtained in all samples positive for Blastocystis on direct microscopy. After DNA extraction PCR was positive for most of the samples.

Conclusion: Blastocystis was present in chronic diarrheal patients either alone or in combination with other parasites. Detection of Blastocystis was greater by culture on Locke-egg media as compared to direct smear and PCR gave good results after DNA extraction of Blastocystis.

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Evaluation of in vitro anti-leishmanial activities of curcumin and its derivatives "gallium curcumin, indium curcumin and diacetylecurcumin"

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Background: *Leishmania* species are intracellular parasitic hemoflagellates that infect macrophages of the skin and viscera to produce diseases in their vertebrates' hosts. Curcumin is the active ingredient in the herbal remedy and dietary spice turmeric (*curcuma longa* linn). Curcumin was identified to be responsible for most of the biological effects of turmeric.

Methods: *Leishmania major* promastigotes were cultivated in RPMI 1640 and each well of micro plate was filled with a final concentration of 4×106 parasites/ml (100μl) of culture medium and after the incubation period, the test agents including curcumin, indium curcumin (In(CUR)3), diacetylcurcumin (DAC) and gallium curcumin (Ga(CUR)3) were added. Negative control only received RPMI medium, and the positive control contained varying concentrations of standard anti-leishmanial compound, amphotericin B. MTT solution was added to each well and incubated at 25°C for 72 hours. Afterward, isopropanol was added for solving the formazan crystals. Finally, the plates were read with an ELISA reader using 540 nm as test wavelength and 630 nm as the reference wavelength.

Results: The IC50 values for curcumin, Ga(CUR)3, In(CUR)3, DAC and amphotericine B were 38μg/ml, 32μg/ml, 26μg/ml, 52μg/ml